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Acute Lung Injury Induced by a Commercial Leather Conditioner

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Following a formulation change, a leather conditioner was involved in a 1992 nationwide outbreak of respiratory illness. We investigated the composition and toxicity of the conditioner produced before (previous product) and after (new product) the disease outbreak. The new product induced tachypnea, pulmonary edema, pulmonary hemorrhage, and sporadic deaths in exposed guinea pigs and rats. Ultrastructurally, these changes were associated with direct pulmonary cytotoxicity characterized by necrosis of alveolar type I cells and alveolar septal interstitial edema. Chemical analyses suggested major alterations in the fluorohydrocarbon constituents in the new formulation of the leather conditioner. While these alterations could not be specifically identified, they appeared to include changes from fluoralkanes to fluoroalkenes, fluorophenyl, and/or fluoroalcohol compounds. Changes in solvent composition were consistent with traces of 2-butoxyethanol and isomers of dipropylene glycol methyl ether, and additional C_{10}–C_{12} alkanes. In this study, we demonstrated the toxicity of the new product in laboratory animals. Some of the altered constituents of the new product have been identified and are potential candidates for additional investigations to identify specific etiologic agents.

In 1992 and 1993, outbreaks of human respiratory illness were associated with exposure to sprays used to treat leather items (Smilkstein *et al.,* 1993; Kulig *et al.,* 1993). Both sprays had been recently reformulated to comply with the 1990 amendments to the Clean Air Act. The 1992 disease outbreak involved primarily respiratory symptoms, including pleuritic chest pain, cough, and shortness of breath. Pulmonary infiltrates were radiographically demonstrated in three patients, and one patient had adult respiratory distress syndrome. Systemic signs, including fever and malaise, were present in some patients. The first cases of this disease appeared on December 23, 1992; the disease was recognized and attributed to the leather conditioner by December 27, 1992, and a voluntary recall began on that same day with completion of the recall by December 31, 1992. Despite the rapid response to a public health emergency, the 1992 outbreak affected people throughout the United States and it has been estimated that more than 500 different individuals displayed adverse responses (Smilkstein *et al.,* 1993). A second, similar disease outbreak was recognized in November, 1993, and attributed to a different shoe spray (Kulig *et al.,* 1993).

We wished to investigate whether toxicity testing in animals might have predicted the human disease outbreak. The outbreak could have been caused by changes in either the use of the leather conditioner or its toxicity. We, therefore, examined the hypothesis that the 1992 disease outbreak was associated with increased toxicity of the new leather conditioner rather than less stringent ventilation procedures accompanying the use of the new product. In examining this hypothesis, we established models of this respiratory disease in guinea pigs and in rats exposed to the new product involved in the 1992 disease outbreak. In addition, we characterized the morphopathogenesis of the experimental disease and related these findings to the clinical findings in affected humans. Finally, we examined the chemical constituency of the leather conditioner. A preliminary report of the results from the first group of experimentally exposed guinea pigs has been included in the epidemiology report of the human disease outbreak (McAnulty *et al.,* 1997). End points examined included breathing rates, bronchoalveolar lavage fluid cytology, and light microscopic as well as ultrastructural changes in the lungs of exposed animals.

MATERIALS AND METHODS

Samples of the leather conditioner. Samples of leather conditioner associated with a disease outbreak (new product) and samples not associated with reported disease (previous product) were received from concerned individuals during an outbreak of respiratory disease (Smilkstein *et al.,* 1993). Specifically, the batches used for both chemical characterization and animal toxicity testing were the batch with the stamp 1092 on the container (previous product) and the batch with the stamp 1292 on the container (new product). These appeared to be date stamps.

Chemical characterization of the leather conditioner. For characterization of volatile components, portions of the products were sprayed into vials and the headspace immediately sampled with sorbent tubes (thermal desorption tubes containing three sorbent beds sampled for 30 sec at 20 cc/min). These thermal desorption tubes were analyzed using a Perkin–Elmer

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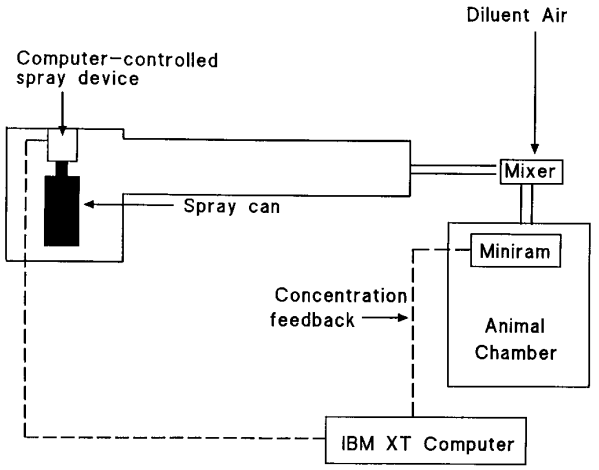

FIG. 1. The leather spray exposure system.

ATD 400 automated thermal desorption system interfaced to a HP5890 gas chromatograph-HP5970 mass spectrometer (GC–MS). A second portion of each sample was dissolved (sprayed) in carbon disulfide and analyzed directly by GC–MS (HP5890GC-HP5971MS). Both of these analyses were performed using 30-m DB-1 capillary columns.

For characterization of fluorohydrocarbons, a 10-μL aliquot of each sample was injected into a glass sampling tube configured for the ATD. These aliquots were allowed to air dry overnight so most of the volatile solvents would evaporate. The glass tubes were then heated at 400°C for 10 min in the ATD unit and analyzed directly by GC–MS.

Experimental animals. Male, specific-pathogen-free, English shorthair guinea pigs (200–250 g; Camm Research Laboratory Animals, Wayne, NJ) and male, specific-pathogen-free, Sprague–Dawley rats (200–250 g; Hilltop Lab Animals, Inc., Scottdale, PA) were housed in laminar flow units and provided water and guinea pig chow or autoclaved rat chow *ad libitum* before and after exposure. Surveillance rats were consistently negative for Sendai, pneumonia virus of mice, sialodacryoadenitis virus/rat coronavirus, Kilham rat virus, Toolan's H-1 virus, reovirus type 3, *Mycoplasma pulmonis,* and Hantaan virus. Surveillance guinea pigs were consistently negative for Sendai, pneumonia virus of mice, reovirus type 3, and lymphocytic choriomeningitis virus but were variably positive for *Encephalitozoon cuniculi* and simian virus-5. Animals were euthanized by intraperitoneal injection of pentobarbital.

The number of animals exposed to the new and the previous product was limited by the available quantity of leather conditioner. The total number of guinea pigs exposed to the high concentration of the previous product, high concentration of the new product, low concentration of the new product, or room air were 20, 28, 8, and 22, respectively. The total number of rats exposed to the new product or room air were 12 and 8, respectively.

Exposure apparatus. The respirable fraction of the leather conditioner was generated by mounting an aerosol spray can in a system which employed a computer-controlled solenoid ''finger'' periodically pressing on the nozzle–valve assembly to modulate spraying time, i.e., a 0.1-sec spray every 15 sec for 2 hr (Fig. 1). All exposures were for a period of 2 hr except for the eight rats designated for necropsy within 1 hr of exposure, in which the exposure was decreased due to high mortality associated with the first 2-hr exposure of rats (Table 2). The count median diameter of the aerosol was 2.7 μm and the count geometric standard deviation was estimated at approximately 4.0 μm [determined with an aerodynamic particle sizer (APS Model 3300, TSI)]. The mass median aerodynamic diameter was 7.8 μm while the peak number diameter was ~0.7 μm. Guinea pigs and rats were exposed in groups of four in a 30-cm-diameter cylindrical chamber that had conical-shaped ends. The mass concentration of the leather spray aerosol within the chamber was monitored continuously with a light scattering monitor (Miniram, Model PDM-3, MIE). Ten-second estimates of the average aerosol mass concentration were used to adjust the flow of diluent air that was mixed with the aerosol prior to its entrance into the animal exposure chamber. In initial experiments, monitor readings of 4.87 (high concentration) and 2.67 (low concentration) were shown to correspond to filter sample calculations of 2.5 ± 0.4 and 1.5 ± 0.2 mg/m^3. In subsequent exposures, the flow of diluent air was adjusted to recreate these exposure conditions (Table 1).

Physiologic measurements. Breathing rate was measured in a glass plethysmograph using a modification of a previously described technique (Ellakkani *et al.,* 1984). The breathing rate was determined first in air and then in a 10% CO_2 atmosphere. Pressure changes, proportional to the animal's breathing pattern, were quantitated by a pressure-sensitive transducer (Setra Model 239). A high-resolution signal analyzer (B&K, Model 2033) calculated the spectral content of the pressure signal, including breathing frequency and harmonics.

For physiologic measurements, guinea pigs and rats were exposed either to air or to leather conditioner for 2 hr. Breathing rates for guinea pigs were obtained immediately, 8 hr, or 18 hr after exposure to leather conditioner (Table 1, Fig. 4). Breathing rates for rats were obtained immediately after exposure to leather conditioner (Table 2).

Harvest and quantitation of free lung cells and lavage protein. Alveolar macrophages, erythrocytes, and pulmonary leukocytes (here defined as granulocytes and lymphocytes) were harvested by bronchoalveolar lavage (BAL) using a calcium and magnesium-free phosphate-buffered solution [145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4, 9.35 mM Na_2HPO_4, and 5.5 mM glucose (pH 7.4)] as previously described (Miles *et al.,* 1978). Briefly, under pentobarbital anesthesia, the trachea was cannulated, and the lung was repeatedly lavaged until 80 ml of lavage fluid was collected (7–8 ml/lavage). Lavage cells were centrifuged, washed, and suspended in buffered Hepes [145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 5.5 mM glucose, and 10 mM Hepes (pH 7.4)].

Differential cell counts were made using an electronic cell counter equipped with a cell-sizing attachment which distinguishes alveolar macrophages, leukocytes (granulocytes and lymphocytes), and erythrocytes (Castranova *et al.,* 1979, 1990; Jones *et al.,* 1980). Protein content of the acellular lavagate from two control guinea pigs and four guinea pigs 8 hr after exposure to the high dose of the new product was determined using the supernatant fluid recovered by centrifugation of the first lavage (4.7 ± 0.35 ml recovered). Protein content was measured by the method of Lowry *et al.* (1951) and expressed as mg protein/ml of BAL fluid.

Pathology. Three pathology experiments were conducted. These were two experiments using guinea pigs and one experiment using rats. Lungs and tracheas were rapidly removed, perfused with 10% neutral-buffered formalin (first guinea pig study) or Karnovsky's (Karnovsky, 1965) solution (second guinea pig study and rat study) at a pressure of 30–34 cm H_2O (at an altitude of 960 ft). For the second guinea pig study, the right middle lung lobe was ligated before perfusion with Karnovsky's solution and was separately perfused with formalin using a 25-gauge needle inserted through the pleura. Remaining tissues were fixed in 10% neutral-buffered formalin. From guinea pigs, the following tissues were trimmed for light microscopic examination: left cranial lung lobe, right cranial lung lobe, right middle lung lobe (second study only), right caudal lung lobe, trachea, adrenal, kidney, liver, and testicles. From rats, the following tissues were trimmed for light microscopic examination: left lung lobe, right cranial lobe, right caudal lobe, trachea, kidney, and liver. Tissues for light microscopy were routinely processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Lung sections for electron microscopy were from the left cranial lobe of guinea pigs (second study only) and the left lung lobe of rats and were postfixed in osmium tetroxide, embedded in Epon, sectioned with a diamond knife, and stained with uranyl acetate and lead citrate as previously described (Lapp *et al.,* 1991).

TABLE 1
Exposure of Guinea Pigs to a Leather Conditioner

Exposure group	Light scattering monitor reading[a]	Number of animals	Number of animals for pathology[b]	Number of animals for cellular assay[b]	Number of animals for breathing rate studies[b,c]
Clean air	N/A[d]	22	10	12	22
Previous product	4.92 ± 0.04	20	8 (4, 4, 0, 0)	12 (4, 4, 0, 4)	20 (20, 8, 0, 4)[d]
New product	2.44 ± 0.24	8	0	8[e] (4, 4, 0, 0)	8 (8, 3, 0, 0)
New product	4.63 ± 0.17	28	16[f] (8, 4, 4, 0)	12[g] (4, 4, 0, 4)	28 (20, 8, 0, 2)

[a] Expressed as the mean ± standard error of the mean of the monitor reading. In three representative high-concentration exposures of the new product, monitor readings of 4.82 ± 0.06 represented filter sample measurements of 3.17 ± 0.33 mg/m^3. In one representative low-concentration exposure of the new product, a monitor reading of 2.67 represented filter sample measurements of 1.5 ± 0.2 mg/m^3. In two representative high-concentration exposures of the previous product, monitor readings of 4.95 ± 0.09 represented filter sample measurements of 3.3 ± 0.4 mg/m^3. All exposures were for 2 hr. In one group of four guinea pigs, a 5-min exposure to the previous product at 10 mg/m^3 followed the 2-hr exposure to the previous product.

[b] Numbers in parentheses are the numbers of animals used immediately after exposure, 8 hr after exposure, 10 hr after exposure, and 18 hr after exposure.

[c] Animals used for breathing rates (Fig. 4) were subsequently used for other experiments.

[d] Not applicable.

[e] One guinea pig died prior to the scheduled sacrifice time.

[f] Three guinea pigs died prior to the scheduled sacrifice time.

[g] Two guinea pigs died prior to the scheduled sacrifice time.

Evaluations of morphologic alterations were conducted by a veterinary pathologist blinded to the exposure status of the animals. Histopathologic alterations from the first guinea pig study were independently confirmed by a second pathologist. Light microscopic evidence of alveolar hemorrhage was scored for lesion severity (none—0, minimal—1, mild—2, moderate—3, marked—4, severe—5) and for distribution (none—0, focal—1, locally extensive—2, multifocal—3, multifocal and coalescent—4, diffuse—5).

Sections of the left cranial lung lobe from guinea pigs euthanized immediately after exposure to the previous and new product were examined for particulate deposition using backscattered scanning electron microscopy and energy dispersive X-ray analysis (Abraham and DeNee, 1974).

Statistics. Statistics were performed using a computerized statistical program (SigmaStat; Jandel Scientific, San Rafael, CA). Comparisons of two groups of normally distributed data were performed using a Student's *t* test. If conditions for validity of the *t* test were not met, a Mann–Whitney rank sum test was used. Multiple comparisons of normally distributed data were made using an analysis of variance. Data which were significantly different in an analysis of variance were further analyzed to determine either (1) which groups differed significantly from control values (Bonferroni method) or (2) which treatment groups differed significantly from all other groups (Student–Newman–Keuls). Multiple comparisons of data which were not normally distributed were made using a Kruskal–Wallis one-way analysis of variance on ranks; comparisons with control values were made using Dunn's method. When differences between control and exposed groups were not significant and power analysis revealed insufficient animal numbers, exposed groups were combined and compared using a Mann–Whitney rank sum test.

TABLE 2
Exposure of Rats to a Leather Conditioner

Exposure groups	Light scattering monitor reading	Number of animals	Number of animals for pathology	Number of animals for cellular assays	Number of animals for breathing rate studies
Clean air	N/A[a]	8	4	4	8
New product	6.1[b]	4[c]	1	0	0[d]
New product	5.2[e]	4[f]	4[f]	0	2[d]
New product	2.2[g]	4[h]	0	4	4

[a] Not applicable.

[b] The reading on the light scattering monitor. The filter sample measurements were 3.2 ± 2.0 mg/m^3. The exposure was for 2 hr.

[c] Three of the four rats in this group died in the exposure chamber. They were not necropsied.

[d] One surviving rat was considered too weak for breathing rate studies.

[e] The reading on the light scattering monitor. The filter sample measurements were 1.9 ± 1.2 mg/m^3. The exposure was for $1\frac{1}{2}$ hr.

[f] One of the four rats in this group died in the exposure chamber.

[g] The reading on the light scattering monitor. The filter sample measurements were 1.5 ± 0.5 mg/m^3. The exposure was for $\frac{1}{2}$ hr.

[h] One of the four rats in this group died after removal from the chamber but before bronchoalveolar lavage.

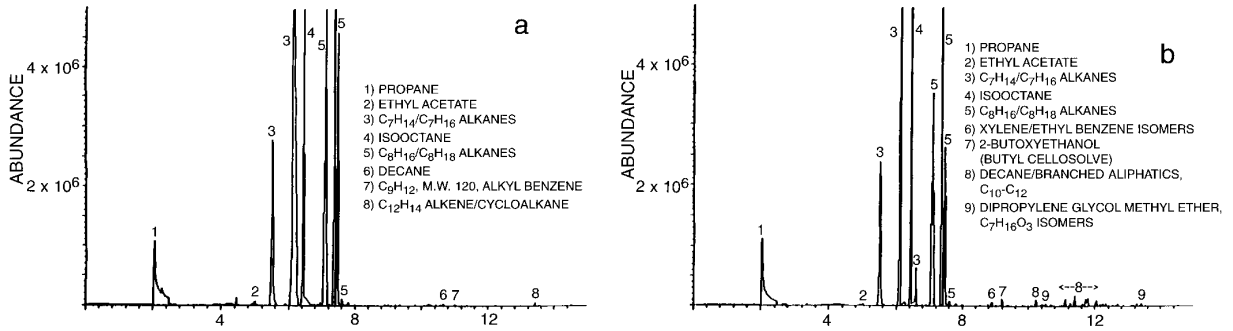

FIG. 2. GC–MS total ion chromatograms of the solvent phase of the leather conditioner involved in the 1992 outbreak of human respiratory disease. These chromatograms are from samples dissolved in carbon disulfide. Similar chromatograms were obtained by headspace analysis after samples were sprayed into vials. (a) The solvent phase of the previous leather conditioner. (b) The solvent phase of the new leather conditioner.

RESULTS

Chemical characterization. The previous and new leather conditioners had similar solvent composition in analyses of both the headspace samples and the solvent solutions. By GC–MS analysis, the major solvent components were propane and C_7–C_8 alkanes with traces of ethyl acetate (Fig. 2). In addition to these compounds, the new product apparently contained traces of 2-butoxyethanol, dipropylene glycol methyl ether isomers, and a few more C_{10}–C_{12} alkanes which were not detected in the previous product.

In the heated samples, fluorohydrocarbons were present in both the previous and the new products. Individual fluorohydrocarbons could not be specifically identified. However, comparison with the mass spectral library suggested the presence of fluoroalkene, fluorophenyl, and/or fluoroalcohol compounds in the new product rather than the fluoroalkanes suggested by the spectra of the previous product (Fig. 3).

Lethal effects of the leather conditioner. Lethality was observed in guinea pigs and rats exposed to the new leather conditioner at both exposure concentrations (Tables 1 and 2). The overall mortality rate was 6 of 36 guinea pigs and 5 of 12 rats exposed to the new leather conditioner.

Time- and concentration-dependent alterations in breathing rate. The baseline breathing rate in 10% CO_2 was significantly elevated immediately after guinea pigs were exposed for 2 hr to the high concentration of new product. Breathing rates in 10% CO_2 were also significantly elevated 8 and 18 hr after exposure; there was no evidence of recovery ($p \leqslant 0.0001$, Kruskal–Wallis one-way analysis on ranks; $p \leqslant 0.05$, Dunn's comparison with control). At all time points, the breathing rates of guinea pigs exposed to the new product exceeded the breathing rate of guinea pigs exposed to the previous product for the same time period ($p \leqslant 0.05$, t test). This tachypnea exhibited some concentration dependence; i.e., the breathing rate 8 hr after a low concentration exposure (195 breaths/min) was approximately midway between the air control value of 136 breaths/min and the breathing rate 8 hr after exposure to the high concentration (252 breaths/min). The previous product did not affect breathing rate (Fig. 4).

Tachypnea was also observed in rats exposed to leather spray. In six surviving leather spray-exposed rats, the breathing rate of 228 ± 20.66 (mean ± SE) breaths/min was significantly greater than the 156.9 ± 30 (mean ±

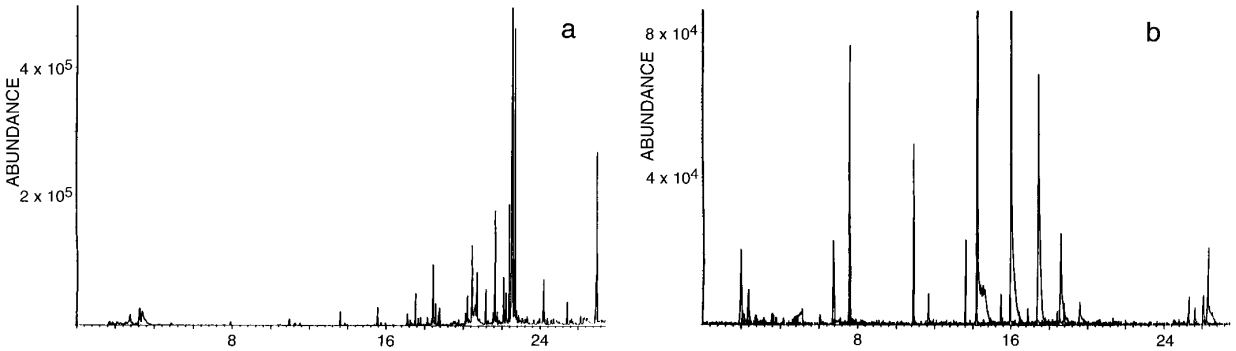

FIG. 3. GC–MS selected ion chromatograms of fluorohydrocarbons present in the leather conditioner involved in the 1992 outbreak of human respiratory disease. (a) Fluorohydrocarbons from the previous leather conditioner. (b) Fluorohydrocarbons from the new leather conditioner.

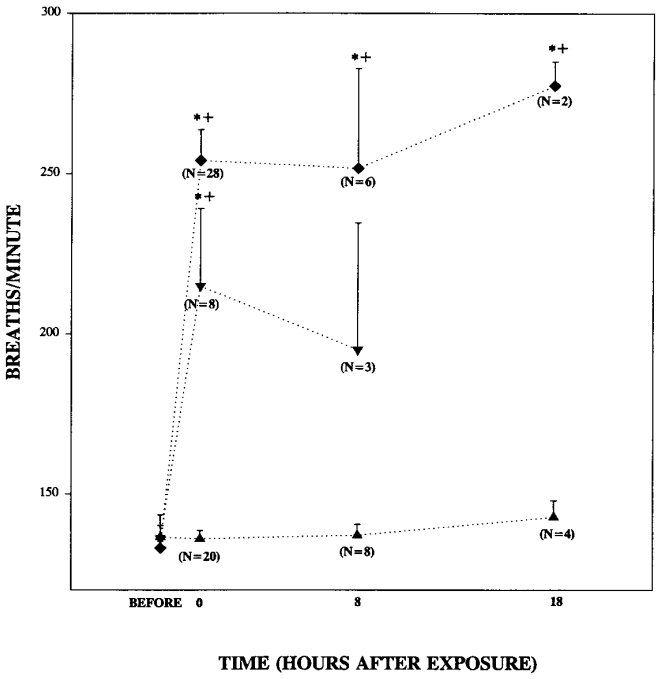

FIG. 4. Breathing rate of guinea pigs in 10% CO_2 versus time after exposure to the high concentration of the previous leather conditioner (▲), a low concentration of the new leather conditioner (▼), or a high concentration of the new leather conditioner (◆). *indicates a significant increase above the preexposed (control) level while + indicates a significant increase above the level after exposure to the previous conditioner ($p \leq 0.05$). Data are the pooled results of seven high-concentration exposures to the new product, two low-concentration exposures to the new product, and five high-concentration exposures to the previous product. Error bars are the standard error of the mean (SEM).

SE) breaths/min seen in controls ($p \leq 0.01$, Mann–Whitney rank sum test).

Alterations in BAL fluid. The principal change in BAL fluid of guinea pigs exposed to a high concentation of leather conditioner was an increase in the number of erythrocytes (Fig. 5). This change was significant at all time points. Protein accompanied the cellular influx with a 1614% increase over control levels, 8 hr after exposure. In the BAL fluid of guinea pigs exposed to the previous product, no elevation in lavage protein or in the numbers of leukocytes (granulocytes and lymphocytes) and erythrocytes was noted; however, there was a significant increase in the number of alveolar macrophages harvested 18 hr postexposure. No significant changes were noted in the cell differentials in BAL fluid of rats immediately following a low-concentration exposure to the new product (data not shown).

Pathology. In both guinea pigs and rats, lesions associated with exposure to the new product were restricted to the lungs. The principal light microscopic lesion was alveolar hemorrhage. An increased severity and extent of hemorrhage in the lungs of rats and guinea pigs exposed to the new product was observed compared to controls (Fig. 6). Alveolar hemorrhage was not seen in guinea pigs exposed to the previous leather conditioner. Alveolar edema was also observed in some animals exposed to the new product in each study, often after a delay. In the first guinea pig study, alveolar edema was seen in one of three guinea pigs surviving 10 hr after exposure and in the one guinea pig that died 9 hr after exposure. In the second guinea pig study, alveolar edema was seen in two of two guinea pigs that died after exposure and in one of two guinea pigs surviving 8 hr after exposure. In the rat study, alveolar edema was seen in the one rat surviving 8 hr after exposure and in one of three rats necropsied within 1 hr of exposure.

Pulmonary inflammation was not a major lesion. In the rat study, two of three rats necropsied within 1 hr of exposure had multifocal, minimal, subpleural, suppurative interstitial pneumonia restricted to the right apical lung lobe. The rat necropsied 8 hr after exposure had mild to moderate, acute, diffuse, suppurative, interstitial pneumonia involving all three lung lobes examined microscopically.

Ultrastructurally, lesions associated with inhalation of the new leather conditioner were interstitial edema, expansion of alveolar epithelial cell intercellular junctions, and necrosis of alveolar type I cells. These ultrastructural alterations were seen in both rats and guinea pigs and were sometimes severe (Fig. 7). However, lesion severity varied dramatically within sections from the same animal, with severe interstitial edema

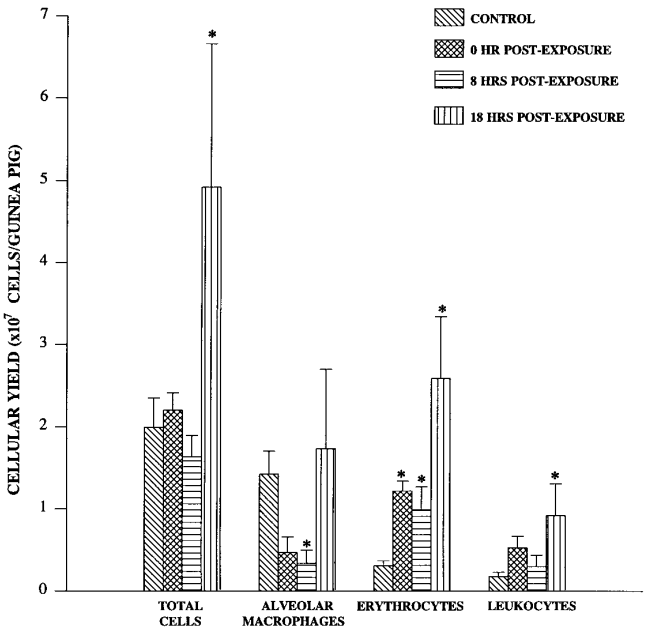

FIG. 5. Cellular changes in the bronchoalveolar lavage fluid of guinea pigs in response to the new leather conditioner. *indicates a significant difference from the room air control ($p \leq 0.05$, one–way analysis of variance; $p \leq 0.05$, Bonferroni comparison with control). Error bars are the SEM.

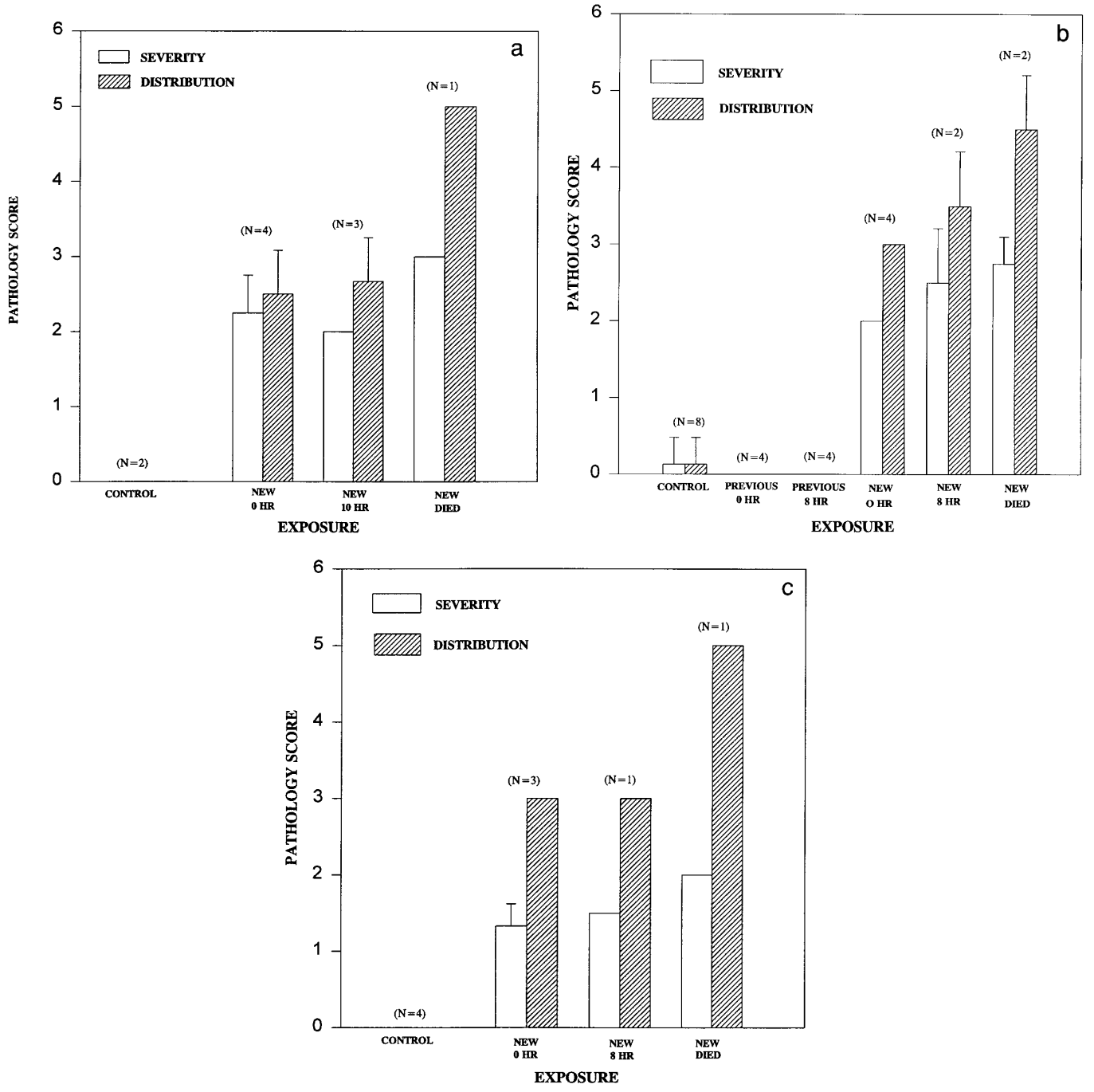

FIG. 6. Quantitative scores of the severity and distribution of hemorrhage in rats and guinea pigs exposed to leather conditioner. Previous refers to the previous formulation; new refers to the new product; control refers to room air controls. Error bars are the standard deviation of the mean. (a) Severity and distribution of hemorrhage in the first guinea pig study. For severity of hemorrhage, the control group is significantly different from the combined groups exposed to the new product ($p = 0.0444$, Mann–Whitney rank sum test). For distribution of hemorrhage, each of the exposed groups was significantly different from controls ($p = 0.00268$, one-way analysis of variance; $p \leq 0.05$, Dunnett's pairwise multiple comparison procedures). (b) Severity and distribution of hemorrhage in the second guinea pig study. For severity and distribution of hemorrhage the combined groups exposed to the new product are significantly different from both the controls and the combined groups exposed to the previous product ($p = 0.0000327$, Kruskal–Wallis one-way analysis of variance on ranks; $p \leq 0.05$, pairwise multiple comparison, Student–Newman–Keuls method). Neither the severity nor the distribution of hemorrhage in the guinea pigs exposed to the previous product differed significantly from control values. (c) Severity and distribution of hemorrhage in rats exposed to the new product. Both the distribution and the severity of hemorrhage in the combined exposed groups differed significantly from control values ($p = 0.0159$ for both severity and distribution, Mann–Whitney rank sum test).

and complete denudation of the lining epithelium seen in some foci from lungs which were unaffected elsewhere.

Particle deposition was not observed in the lungs by backscattered examination.

DISCUSSION

The leather conditioner associated with a 1992 outbreak of human respiratory disease (Smilkstein *et al.,* 1993) was highly toxic to guinea pigs and rats. We devised the concentrations used for these animal studies from the anticipated maximum exposures for people using the leather conditioner in a poorly ventilated area. In both rats and guinea pigs, physiological measurements of tachypnea were consistent with reports of shortness of breath and/or tachypnea seen clinically in humans exposed to leather products in similar disease outbreaks (Smilkstein *et al.,* 1993; Kulig *et al.,* 1993). Histopathologic examination of lungs from guinea pigs and rats demonstrated pulmonary hemorrhage and edema following exposure to the new product but not following exposure to the previous product. These findings were confirmed by analysis of BAL in the guinea pig study. These morphologic changes in exposed animals may relate to the infiltrates observed in radiographs of affected humans (Smilkstein *et al.,* 1993; Kulig *et al.,* 1993). Ultrastructurally, the destruction of the pulmonary air/capillary barrier was associated with interstitial edema and necrosis of the alveolar epithelium. These findings correlate with increases in BAL protein and red blood cells in guinea pigs following inhalation of the new product.

Background, nonsuppurative interstitial inflammatory lesions in guinea pigs, not associated with exposure, may have prevented the detection of subtle inflammatory lesions in this species (Percy and Barthold, 1993). Suppurative inflammation was associated with leather spray exposure in three of the four rats which survived the exposure. However, pulmonary inflammation was only an important microscopic feature in the one rat which survived for 8 hr. The early time points examined in these studies may not have permitted the complete development of acute inflammatory lesions (Slauson and Cooper, 1990).

The concentrations of leather conditioner used in our study resulted in an unanticipated lethal effect in some of the experimental animals which was not seen in the outbreak of human disease. This may have been a function of exposure dose. The experimental conditions (e.g., 0.1 sec of spray every 15 sec for 2 hr) may have produced higher concentrations or a longer duration of exposure than occurred during the outbreak among humans. Importantly, some people reported symptoms within minutes of exposure (Smilkstein *et al.,* 1993) and may have avoided additional exposures. Individual and species differences in susceptibility may also have contributed to the lethal response to the concentrations used in these studies.

Specific changes in the composition of this leather conditioner during reformulation were reported to be changes in the solvents, propellants, and fluoropolymers. In a different outbreak, involving a leather spray produced by another manufacturer, different compounds were used for the solvents and propellants (Kulig *et al.,* 1993). The literature on the composition of the fluorocarbons is contradictory, with one report that the fluoropolymers in the leather conditioner involved in the 1992 outbreak were changed from 1% FC-905 to 1.2% FC-3537 (Smilkstein *et al.,* 1993) and another report that in both the 1992 and the 1993 outbreaks the fluoropolymers were changed to FS-4565 (Kulig *et al.,* 1993). Chemical analyses conducted as part of our study suggest major differences in the fluorohydrocarbon constituents of the two batches studied, specifically an apparent change from fluoroalkanes to fluoroalkenes, fluorophenyl, and/or fluoroalcohol groups.

Importantly, while fluoroalkanes are generally of low toxicity, some fluoroalkenes are highly toxic (Clayton, 1977). The toxicity has been attributed to electron sharing between the halide and nearby double bonds. The result of electron sharing is a tendency for these compounds to become electrophilic with potential attack on cellular nucleophiles. The pulmonary hemorrhage, edema, and alveolar type I cell necrosis seen in our study are similar to morphologic alterations associated with exposure to perfluoropolymer fumes (polymer fume fever) (Warheit *et al.,* 1990; Lee and Seidel, 1991). In addition, pulmonary edema and hemorrhage are seen following exposure to perfluoroisobutylene, the most toxic of the fluoroalkenes (Clayton, 1977). Interstitial edema and alveolar type I cell necrosis have also been observed in the lungs of rats exposed to perfluoroisobutylene (Brown and Rice, 1991). While the specific features of pulmonary toxicosis induced by the new leather conditioner are alveolar edema, pulmonary hemorrhage, alveolar interstitial edema, and alveolar type I cell necrosis, the morphologic lesions observed are not unique. They are seen as a result of direct cytotoxicity of several pulmonary toxicants but are also characteristic of a general category of pulmonary responses to inhaled toxicants (Menzel and McClellan, 1980).

Since the previous product investigated in our study contained only traces of 1,1,1-trichloroethane, the solvent eliminated to comply with the 1990 Amendments to the Clean Air Act, it is possible that both the previous and the new products represent reformulations of a 1,1,1-trichloroethane-containing product. The presence of more than one reformulated product may also explain the conflicting literature on the fluorohydrocarbon composition after reformulation. Our findings are also consistent with distinctly different chemical and toxic properties of the previous and new products.

Chemical differences between the new and the previous products appear to include the presence of 2-butoxyethanol and isomers of dipropylene glycol methyl ether and increases

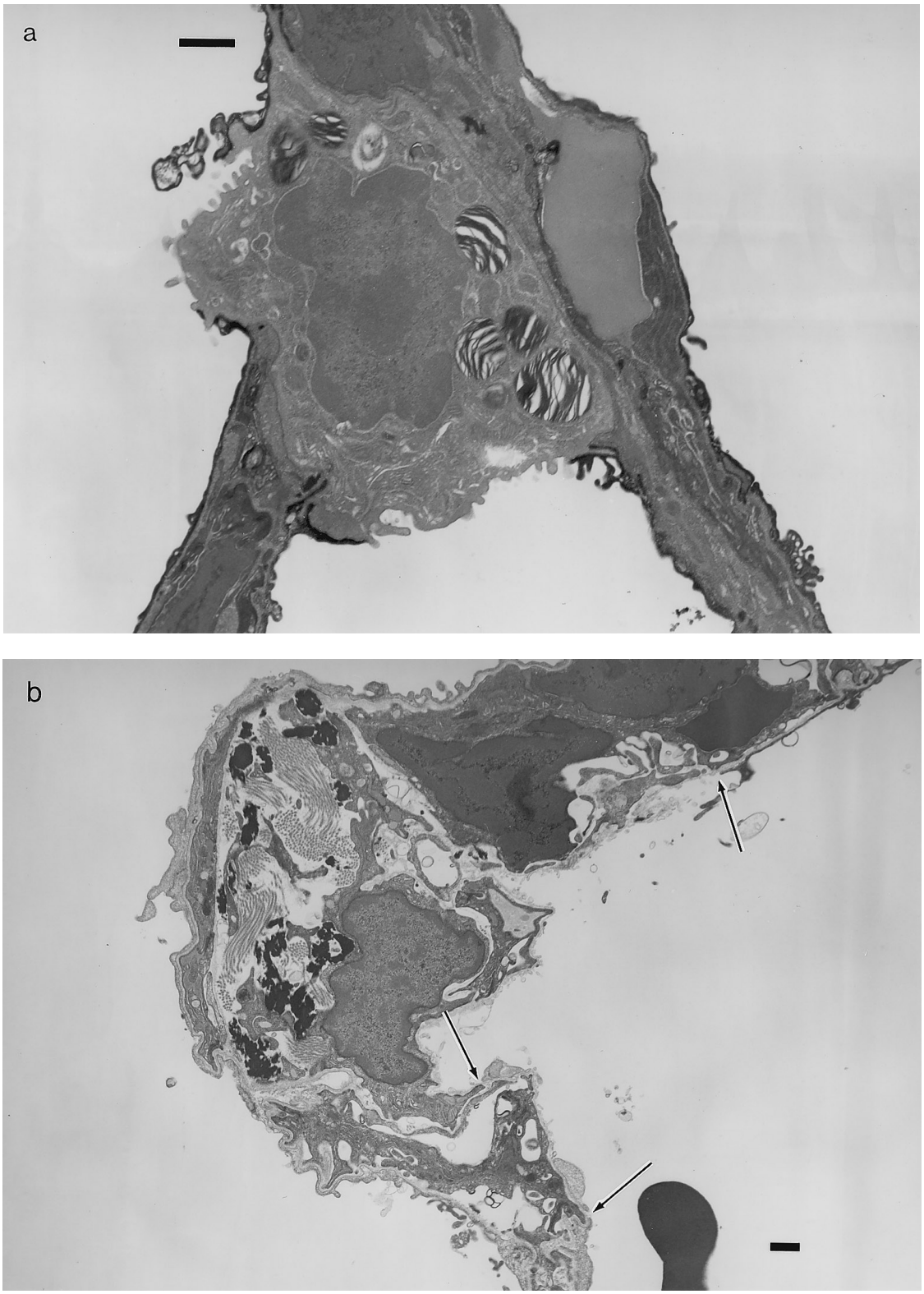

FIG. 7. Ultrastructural alterations following exposure to leather conditioner. (a) Control rat. Bar, 1 μm. (b) Rat exposed to the new product and examined within 1 hr of exposure. The interstitium is markedly edematous and the alveolar epithelial surface is multifocally devoid of alveolar epithelial cells. Arrows indicate denuded basement membrane. Bar, 1 μm. (c) Rat exposed to the new product and examined 8 hr after exposure. The interstitium is diffusely, severely edematous. Focally, the alveolar surface is denuded of epithelial cells (small arrows). Large arrow indicates cytoplasmic projection of a type I cell. Bar, 1 μm.

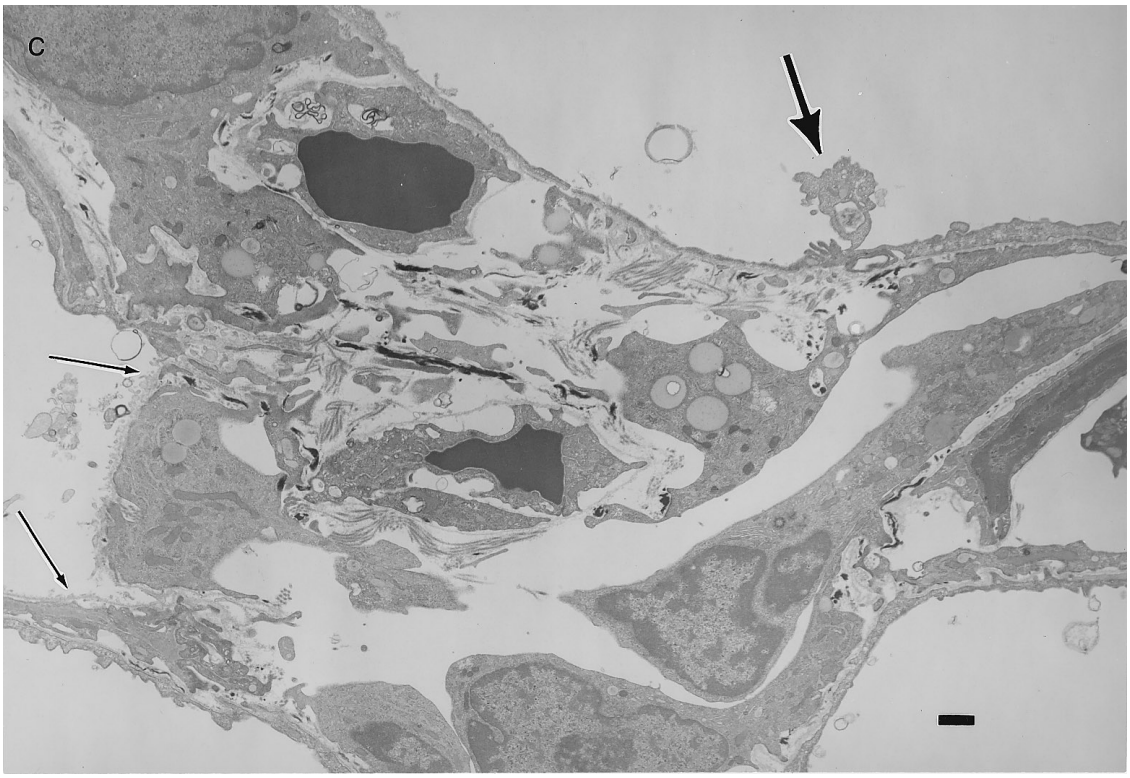

FIG. 7—*Continued*

in $C_{10}-C_{12}$ alkanes in addition to changes in the fluorohydrocarbons (Smilkstein *et al.*, 1993; Kulig *et al.*, 1993). Interestingly, one of the chemicals in the new product, 2-butoxyethanol, is more toxic to rats than to guinea pigs (Browning, 1965) and more toxic to laboratory animals than to humans (Clayton and Clayton, 1981–1982)—the same order of toxicity that occurred in our investigation of the new conditioner. However, 2-butoxyethanol is not a known cause of the pulmonary lesions seen in our study and is associated with hemolysis and renal lesions not observed in our study (Cornish, 1980). It is also possible that interactions between the agents present in the new product may have produced an unanticipated toxic effect. For example, aliphatic compounds can act as defatting agents and, by altering pulmonary permeability, potentially alter the toxicity of other constituents in the new product (Cornish, 1980). Thus, additional investigation of the components present in the new product are indicated to prevent another similar outbreak.

Recently, changes in constituents in sprays used in the textile industry have resulted in severe interstitial lung disease with fatalities in Algeria and Spain (Moya *et al.*, 1994; Kadi *et al.*, 1994). Thus, enhanced toxicity associated with altered ingredients is not unique to this leather conditioner. In the case of the leather conditioner, reformulation was initially intended to reduce ozone-depleting components. Ironically, the attempt to be environmentally progressive resulted in adverse health effects.

This investigation is a unique opportunity to compare toxicity in animals and humans. The toxicity of the new leather conditioner is demonstrable in two different laboratory animal species, rats and guinea pigs. The previous product, which is not a known cause of human disease, does not produce significant changes in laboratory animals. The new product, which has been associated with a nationwide outbreak of human disease, causes tachypnea, pulmonary hemorrhage, and death in laboratory animals. This investigation supports the relevance of animal toxicity studies to human toxicosis. Our findings are consistent with increased toxicity of the new product and suggest that the human disease outbreak cannot be explained solely on the basis of differences in ventilation during application. In laboratory animals, the toxicity of the new leather conditioner is attributable to direct pulmonary cytotoxicity.

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